Acetic Acid (K-ACETGK)
Procedure for ChemWell® 2910 Auto-Analyser

Requirements:

- Acetic Acid GK Assay Kit (K-ACETGK) (provides ~ 500 assays).
- K-ACETGK ChemWell® 2910 assay file.
- Use in association with the Acetic Acid GK Assay Kit (K-ACETGK) product data booklet.

Use:

For the specific measurement of acetic acid (acetate) especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the Acetic Acid GK Assay Kit (K-ACETGK) data booklet.

Assay Principle:

Conversion of acetic acid via the following reactions is directly proportional to the coupled formation of NADH:

(1) Acetic acid + ATP $\xrightarrow{\text{(AK)}}$ acetyl-phosphate + ADP

(2) Acetyl-phosphate + CoA $\xrightarrow{\text{(PTA)}}$ acetyl-CoA + P_i

(3) ADP + D-glucose $\xrightarrow{\text{(ADP-GK)}}$ glucose 6-phosphate + AMP

(4) Glucose 6-phosphate + NAD^+ $\xrightarrow{\text{(G6P-DH)}}$ 6-phosphogluconate + NADH + H^+

Procedure:

Prepare the assay reagents and calibrators and use with the K-ACETGK ChemWell® 2910 assay file.

Acetic Acid GK Assay Kit Components:

Bottle 1: Buffer (11 mL, pH 7.4).
Stable for > 2 years at 4°C.

Bottle 2: NAD^+, ATP, D-glucose, CoA and PVP.
Freeze dried powder.
Stable for > 5 years at -20°C.

Bottle 3: Acetate kinase, phosphotransacetylase, ADP-glucokinase plus glucose-6-phosphate dehydrogenase suspension (2.6 mL).
Stable for > 2 years at 4°C.

Preparation of Kit Components:

1. Use the contents of bottle 1 as supplied.
   Stable for > 2 years at 4°C.
2. Dissolve the contents of bottle 2 in 11 mL of distilled water. **This is Reagent 2 and is stable for > 1 week at 4°C or > 2 years at -20°C** (this reagent is stable when subjected to freeze / thaw cycles, however to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
3. Use the contents of bottle 3 as supplied.
   Swirl the bottle to mix contents before use.
   Stable for > 2 years at 4°C.

Preparation of Assay Reagents: *(per ~ 500 assays)*

Reagent 1:

Component	Volume
distilled water bottle 1 (buffer) bottle 3 (AK/PTA/ADP-GK/G6P-DH)	87.5 mL 10 mL 2.5 mL
Total volume	100 mL

Reagent 1 stability: > 30 days at 4°C / > 2 years at -20°C

Reagent 2:

Component	Volume
*bottle 2 (NAD/ATP/D-glucose/CoA/PVP)	11 mL
Total volume	11 mL

*after adding 11 mL of distilled water

Reagent 2 stability: > 7 days at 4°C / > 2 years at -20°C

Calibrators:

K-ACETGK 1: 0 g/L (use distilled water)
K-ACETGK 2: 0.45 g/L acetic acid
K-ACETGK 3: 0.9 g/L acetic acid
K-ACETGK 4: 1.8 g/L acetic acid

Assay Parameters:

Assay volumes:	Reagent 1: 0.200 mL Sample: 0.003 mL Reagent 2: 0.020 mL
Calibrators:	0, 0.45, 0.9, 1.8 g/L acetic acid
Reaction time:	5 min at 37°C
Wavelength:	340 nm
Assay type:	endpoint
Reaction direction:	increase
Linearity:	up to 1.8 g/L of acetic acid

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